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## (54) COLLAGEN DECOMPOSITION PROMOTER

## (57)Abstract:

PURPOSE: To obtain a collagen decomposition promoter capable of inducing decomposition of the collagen matrix of a connective tissue excessively accumulated in the tissue and useful for therapy of fibrosis diseases.

CONSTITUTION: This collagen decomposition promoter contains one or more kinds of HGFs (hepatocyte growth factor) as the active components. As the HGFs, e.g. HGF is used. HGF can be obtained by extracting an organ, hemocyte, plasma, serum, etc., of a mammal and purifying it or by culturing a primary culture cell or an established cell respectively producing HGF and conducting separation and purification from the products or by a gene engineering technique. The HGFs have a therapeutic effect on fibrosis diseases including arterial sclerosis, chronic glomerular nephritis, dermal keloplasty, progressive systemic sclerosis, fibroid lung, hepatic fibrosis, cystic fibrosis, chronic graft versus host diseases, hidebound diseases (local or systemic), Peyronie's disease, penile fibrosis, urethrostenosis after cystoscopy, internal adhesion after surgical operation, myelofibrosis and idiopathic retroperitoneal fibrosis.

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CLAIMS

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[Claim(s)]

[Claim 1] The collagenolysis accelerator characterized by the thing of HGF for which a kind is contained as an active principle at least.

[Claim 2] The collagenolysis accelerator according to claim 1 which contains HGF as HGF.

[Claim 3] The fibrosing disease failure therapy agent characterized by the thing of HGF for which a kind is contained as an active principle at least.

[Claim 4] The fibrosing disease failure therapy agent according to claim 3 characterized by being chosen out of the group which the above-mentioned fibrosing disease failure becomes from arteriosclerosis, the chronic glomerulonephritis, skin keloid generation, progressive systemic sclerosis (PSS), hepatic fibrosis, the fibroid lung, the cystic fibrosis, the chronic transplant disease for a host, the scleroderma (a part and whole body), a pay RONI Mr. disease, the fibrosing disease of a phallus, the urethrostenosis after the cystoscopy, the internal adhesion after a surgical operation, the myelofibrosis, and an idiopathic posterior part peritoneum fibrosing disease.

[Claim 5] The fibrosing disease failure therapy agent according to claim 3 or 4 which contains HGF as HGF.

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**DETAILED DESCRIPTION**

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**[Detailed Description of the Invention]**

**[0001]**

**[Industrial Application]** This invention relates to a collagenolysis accelerator and a fibrosing disease failure therapy agent. It is related more with a detail at the collagenolysis accelerator and fibrosing disease failure therapy agent which made HGF (Hepatocyte Growth Factor) the active principle.

**[0002]**

**[Description of the Prior Art]** A fibrosing disease is a disease characterized by too much are recording of a connective tissue component, and it is the collagen which the core should be made in a fibrosing disease and should be observed most. It is generated in various internal organs, for example, are recording of a collagen is produced in symptoms like hepatic fibrosis in the fibroid lung and liver in lungs. Moreover, also in the skin, it is generated in symptoms like skin keloid generation, for example. In many cases, are recording of the net of the collagen in a fibrosing disease is a result out of balance between the factors which cause production and decomposition of a collagen. Although various medication had been performed in order to treat the disease and failure of a fibrosing disease, generally they were not what aimed at that the symptomatic therapy of a failure canceled the imbalance in the metabolic turnover factor which adjusts production and decomposition of the pathology, i.e., a collagen and others, which is a core and is the basis of a connective tissue component. So, there was especially nothing that is confirmed in respect of an improvement of an organization into these cures. namely, -- for example, local corticosteroid uses it for treating the inflammation phase in early stages of skin keloid generation -- having -- such [ in the phase of the fibrosing disease of an anaphase like / when / a certain / keloid actually generates as a result of too much collagen production / although an extent success was carried out ] a steroid -- most -- or it is completely ineffective. Thus, with the conventional technique, an approach which a human fibrosing disease failure is treated by the safe and effective approach, generation of the organization of the fibrosing disease beyond it is prevented, and the focus of the fibrosing disease already generated is decreased, or is removed completely was not able to be found out.

**[0003]**

**[Problem(s) to be Solved by the Invention]** The technical problem which this invention tends to solve is to offer the collagenolysis accelerator which can guide decomposition of the collagen matrix of an affinity organization in an in-house accumulated too much, and a therapy agent useful for the therapy of a fibrosing disease failure.

**[0004]**

**[Means for Solving the Problem]** In order to solve the above-mentioned technical problem, as a result of repeating examination wholeheartedly, this invention person etc. has the operation to which HGF promotes decomposition of a collagen, and completed a header and this invention for it being effective in the therapy of a fibrosing disease failure based on the operation. That is, this invention is a collagenolysis accelerator characterized by the thing of \*\*HGF for which a kind is contained as an active principle at least.;

**\*\*** It is related with fibrosing disease failure therapy agent; characterized by the thing of HGF for which a kind is contained as an active principle at least.

**[0005]** In this invention, HGF says the protein in which hepatocyte growth activity is shown, for example, HGF (Hepatocyte Growth Factor) etc. is mentioned. If refined as HGF used by this

invention by extent which can be used as physic, what was prepared by various approaches can be used. Various kinds of approaches are learned as the preparation approach of HGF. For example, it can extract and refine and can obtain from blood cells, such as organs, such as the liver of mammals, such as a rat, a cow, a horse, and a sheep, a spleen, a lung, bone marrow, a brain, the kidney, and a placenta, a platelet, and a leucocyte, plasma, a blood serum, etc. (reference, such as FEBS Letters, 224, 312, 1987, Proc.Natl.Acad.Sci.USA, 86, 5844, and 1989). Moreover, the primary culture cell and established cell line which produce HGF can be cultivated, separation purification can be carried out from cultures (a culture supernatant, cultured cell, etc.), and HGF can also be obtained. Or recombination HGF which inserts in a suitable vector the gene which carries out the code of HGF by the gene engineering-technique, inserts a nest and this in a suitable host, and carries out a transformation and which is made into the purpose from the culture supernatant of this transformant can be obtained (for example, reference, such as Nature, 342, 440, 1989, Biochem.Biophys.Res.Comm., 163, 967, and 1989). Especially the above-mentioned host cell is not limited, but can use various kinds of host cells used by the gene engineering-technique from the former, for example, Escherichia coli, a Bacillus subtilis, yeast, mold, vegetation, or an animal cell. [0006] As an approach of carrying out extract purification of HGF from a body tissue, a carbon tetrachloride can be injected intraperitoneally to a rat, the liver of the rat changed into the hepatitis condition can be extracted and ground, and, more specifically, it can refine in the usual protein purification methods, such as gel column chromatographies, such as S-sepharose and heparin sepharose, and HPLC, for example. Moreover, using the modifying-gene method, by the expression vector which included the gene which carries out the code of Homo sapiens's HGF amino acid sequence in vectors, such as a bovine papilloma virus DNA, the transformation of an animal cell, for example, a Chinese hamster ovary cell (CHO) cell, mouse C127 cell, the ape COS cell, etc. can be carried out, and it can obtain from the culture supernatant.

[0007] as long as HGF obtained in this way is these effects as substantially as HGF -- that, and a part of other amino acid sequences are inserted, or 1 or two or more amino acid have combined with the amino terminal and/or the C terminal \*\*\*\* -- or a sugar chain -- the same -- deletion -- or you may permute. [ that a part of the amino acid sequence is permuted by deletion or other amino acid ]

[0008] The collagenolysis accelerator of this invention makes above-mentioned HGF an active principle, and HGF has the operation which promotes decomposition (increment in collagenase activity) of a collagen, as shown in the example of the after-mentioned trial. Therefore, it is useful also to its prevention besides the therapy of the following fibrosing disease failure, and useful to a therapy and prevention of the disease to which collagenase activity fell, for example, the osteopetrosis etc. Moreover, the fibrosing disease failure therapy agent of this invention is useful for the therapy of the fibrosing disease failure characterized by too much fibroblast production of the affinity organization matrix which makes above-mentioned HGF an active principle similarly, and contains a collagen, fibronectin, and glycosaminoglycan (GAG). The following failure is included by this.

[0009] Arteriosclerosis, the chronic glomerulonephritis, skin keloid generation, progressive systemic sclerosis (PSS), hepatic fibrosis, the fibroid lung, the cystic fibrosis, the chronic transplant disease for a host, the scleroderma (a part and whole body), a pay RONI Mr. disease, the fibrosing disease of a phallus, the urethrostenosis after the cystoscopy, the internal adhesion after a surgical operation, the myelofibrosis, an idiopathic posterior part peritoneum fibrosing disease [0010] The collagenolysis accelerator of this invention and a fibrosing disease failure therapy agent are applied to the collagenolysis promotion and the fibrosing disease failure therapy in others and mammals (for example, a cow, a horse, Buta, a sheep, a dog, a cat, etc.). [ Homo sapiens ]

[0011] Although the collagenolysis accelerator of this invention and a fibrosing disease failure therapy agent can take various formulation (for example, liquids and solutions, a solid preparation, a capsule, etc.), let them be injections, inhalations, suppositories, or an oral agent with the support of it and common use of HGF which are generally an active principle. The injections concerned can be prepared with a conventional method, for example, HGF can be filtered with a filter etc., after dissolving in suitable solvents (for example, the sterilized water, the buffer solution, a physiological saline, etc.), and it can sterilize, and they can be prepared by filling up a sterile container subsequently. As an HGF content in injections, it is usually preferably adjusted to 0.001 to 0.1 (W/V

%) extent 0.0002 to 0.2 (W/V %) extent. Moreover, as an oral medicine, it is pharmaceutical-preparation-ized by dosage forms, such as a tablet, a granule, a fine grain agent, powder, \*\* or hard capsules, liquids and solutions, an emulsion, suspension, and syrups, and these pharmaceutical preparation can be prepared according to the conventional method of pharmaceutical-preparation-izing, for example. Suppositories can also be prepared with the conventional method on the pharmaceutical preparation (for example, cacao butter, the Rau phospholipid, glycerogelatin, macro gall, WITEPPUZORU, etc.) using the basis of common use. Moreover, inhalations can also be prepared according to the stock-in-trade on pharmaceutical preparation. The HGF content in pharmaceutical preparation can be suitably adjusted according to dosage forms, an application disease, etc.

[0012] On the occasion of pharmaceutical-preparation-izing, a stabilizing agent is added preferably and albumin, a globulin, gelatin, a glycine, a mannitol, a glucose, a dextran, a sorbitol, ethylene glycol, etc. are mentioned as a stabilizing agent, for example. Furthermore, the pharmaceutical preparation of this invention may contain an additive required for pharmaceutical-preparation-izing, for example, an excipient, the solubilizing agent, the antioxidant, the aponia-ized agent, the isotonicizing agent, etc. When it considers as liquid preparations, it is desirable for cryopreservation or freeze drying to remove moisture and to save. lyophilized products -- business -- it is used for it, sometimes adding distilled water for injection etc. and sometimes remelting.

[0013] The collagenolysis accelerator of this invention and a fibrosing disease failure therapy agent may be prescribed for the patient according to the suitable route of administration according to the formulation. For example, it can be made the gestalt of injections and a vein, an artery, hypodermically, intramuscular, etc. can be medicated. Although the dose is suitably adjusted by a patient's symptom, age, weight, etc., as HGF, it is 1mg - 100mg preferably, and it is usually appropriate for it to prescribe [ 0.05mg - 500mg ] this for the patient in 1 time per thru/or several steps day.

[0014]

[Effect of the Invention] HGF which are the active principle of this invention can promote and have decomposition (increment in collagenase activity) of a collagen, and it can treat a fibrosing disease failure effectively.

[0015]

[Example] Hereafter, although this invention is explained more to a detail based on the example of manufacture, and an example, this invention is not limited to these examples.

Example of production of example of manufacture 1HGF pharmaceutical preparation (1) HGF 20microg human serum albumin The 100mg above-mentioned matter was dissolved by PBS of 0.01M of pH7.0, the whole quantity was prepared to 20ml, 2ml was poured distributively into each vial bottle after sterilization, and freeze-drying seal was carried out.

(2) HGF 40microg Tween 80 1mg human serum albumin The 100mg above-mentioned matter was dissolved in the physiological saline for injection, the whole quantity was prepared to 20ml, 2ml was poured distributively into each vial bottle after sterilization, and freeze-drying seal was carried out.

[0016] Fibrosis depressant action and a symptom improvement-effect 1. test-method \*\* use animal of HGF to an example 1 dimethylnitrosamine liver fibrosis rat: The Wister system male rat and 5 weeks old \*\* test scheduling dimethylnitrosamine (DMN) were injected intraperitoneally over four weeks every week by the dosage of 10microg/kg on fire, water, and Thursday. HGF administered 500microg/kg intravenously between 28 days (1000microg/kg / day) of bis dice from the time of DMN first time administration (following administration schedule 1 reference). The following measurement was presented with the trial rat on the 29th.

[0017]

投与スケジュール 1



[0018] \*\* The measurement rat was dissected and liver weight was measured. Moreover, the

hydroxyproline content (Hyp; index of fibrosis) and collagenase (collagen dialytic ferment) activity in a hepatic tissue were measured by Kivirikko's and others approach (Anal.Biochem, 19, 249, 1967), and Murawaki's and others approach (J.Biochem, 108, 241, 1990), respectively. furthermore, DNA and the protein content in a hepatic tissue -- respectively -- Dishe -- it measured with the strange method (Biochem, J, 62, 315, 1956) and protein assay kit (biotechnology rat company make) by Burton of law. The result is shown in Table 1. Moreover, the Hitachi 7150 mold automatic analyzer analyzed clinical biochemistry inspection of the blood serum which collected blood from back vena cava to coincidence, and was extracted. The blood test measured a platelet count, a white blood cell count, a number of red cell, a hematocrit value, and hemoglobin concentration using multi-item automatic blood cell counters (E-4000, Sysmex) by the EDTA blood extracted from back vena cava. Moreover, plasma coagulation ability (coagulation time by the prothrombin time, the amount of fibrinogens, and a HEPAPURASUCHIN test and a thrombo test) was measured using the automatic coagulation ability measuring device (KC-40) with the plasma which mixed 3.8% sodium-citrate water solution and the blood extracted from back vena cava at a rate of 1:9. The result is shown in Table 2.

[0019]

[Table 1]

表 1

	溶媒投与群	HGF投与群	健常動物
肝重量 (g)	9.33±0.72	13.02±0.53 **	13.59±0.51 **
総DNA量 (ng/肝臓)	33.6±2.5	39.3±1.8 *	44.8±1.7 **
総蛋白質量 (g/肝臓)	1.36±0.12	1.84±0.09 **	2.33±0.09 **
コラゲナーゼ活性 ( $\mu$ g/min/g-肝臓)	0.22±0.01	0.36±0.07 **	0.27±0.02
ヒドロキシプリン含量 ( $\mu$ g/g-肝臓)	423.1±35.9	300.1±18.0 **	129.3±6.4 **

平均値±標準誤差 (n=10)

\* : P&lt;0.05, \*\* : P&lt;0.01 溶媒投与群との有意差

[0020]

[Table 2]

表 2

血 液 生 化 学 検 査 値	溶媒投与群	HGF投与群	健常動物
GOT (IU/l)	136±9	78±4 **	64±3 **
GPT (IU/l)	50±4	28±1 **	15±1 **
γ-GTP (IU/l)	4.8±0.4	3.4±0.2 **	1.8±0.1 **
総ビリルビン (mg/dl)	0.45±0.08	0.25±0.01 **	0.18±0.01 **
直接型ビリルビン (mg/dl)	0.20±0.02	0.18±0.00	0.13±0.01 **
総蛋白 (g/dl)	4.8±0.1	6.4±0.1 **	5.7±0.0 **
アルブミン (g/dl)	2.4±0.1	3.1±0.1 **	2.6±0.0 **
血糖値 (mg/dl)	131±4	152±6 **	180±8 **
総コレステロール (mg/dl)	53±2	98±5 **	72±3 **
HDL-コレステロール (mg/dl)	28.0±1.9	61.6±3.8 **	41.3±1.7 **
トリグリセリド (mg/dl)	70±9	152±15 **	157±16 **
リン脂質 (mg/dl)	116±4	208±8 **	160±5 **
βリポ蛋白 (mg/dl)	107±11	222±20 **	202±18 **
血 液 ・ 凝 固 検 査 値	溶媒投与群	HGF投与群	健常動物
血小板数 (10 <sup>4</sup> /μl)	31±5	78±4 **	105±5 **
白血球数 (10 <sup>4</sup> /μl)	144±6	101±8 **	87±8 **
赤血球数 (10 <sup>4</sup> /μl)	667±16	702±8 **	755±6 **
ヘマトクリット値 (%)	39.9±0.9	41.6±0.3 *	44.3±0.3 **
ヘモグロビン濃度 (g/dl)	12.7±0.3	13.6±0.1 **	14.6±0.1 **
プロトロンビン時間 (sec)	15.6±0.5	13.8±0.2 *	13.8±0.2 *
フィブリノーゲン (g/dl)	1.45±0.10	2.05±0.11 **	2.31±0.05 **
ヘパプラスチン時間 (sec)	37.3±2.7	28.6±0.6 **	28.5±0.5 **
トロンボテスト時間 (sec)	30.0±1.9	22.5±0.3 **	22.8±0.3 **

平均値±標準誤差 (n=10)

\*: P&lt;0.05, \*\*: P&lt;0.01 溶媒投与群との有意差

[0021] 2. As shown in a result table 1 and Table 2, by the repeated-dose administration of DMN, by the solvent administration group (contrast), expansion and withering of the fibrosis of a remarkable liver were observed and the fall of a liver function clear from clinical biochemistry, blood, and a coagulation system inspection value was accepted. On the other hand, the liver function test value of an HGF administration group showed the value near a healthy animal with the difference more significant than those values of a solvent administration group, and showed the clear improvement effect. Moreover, DNA in a hepatic tissue, the amount of proteins, and collagenase activity rose intentionally by HGF administration, the hydroxyproline content which is the index of fibrosis fell intentionally, and liver weight was mostly recovered to normal level.

[0022] It administered orally to the operation Wister system male rat (6 weeks old) of HGF to an example 2 carbon-tetrachloride liver fibrosis rat to the moon, the carbon tetrachloride was administered orally to Thursday for 12 weeks by the dosage of 0.7 ml/kg every week, and the liver fibrosis model was created. Two following trials were presented with this carbon-tetrachloride liver fibrosis rat.

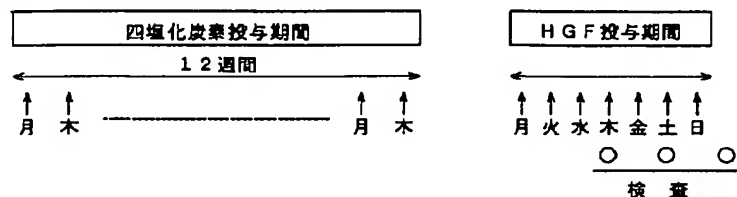
[0023] \*\* HGF was administered intravenously to the carbon-tetrachloride liver fibrosis rat (one groups [ 13-14 ]) of the trial A repeated-dose administration trial above by 50 and 500microg/kg between seven days (100, and 1000microg/kg / day) of bis dice. GOT in the blood serum on 3, 5, and



the 7th, GPT, and a  $\gamma$ -glutamyl transpeptidase value change were compared with the solvent administration group (contrast) from HGF administration initiation (following administration schedule 2 reference). The result is shown in drawing 1. As shown in drawing 1, the recovery promotion operation was accepted from the 7th on 100microg/kg / day in 1000microg/kg / day on the 5th.

[0024]

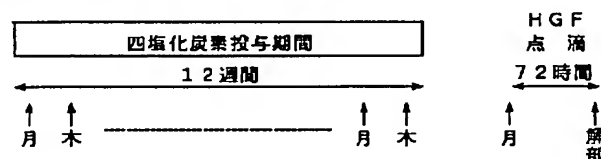
#### 投与スケジュール 2



[0025] \*\* Using the above-mentioned carbon-tetrachloride liver fibrosis rat of trial B instillation trial each 12-13 groups, from the catheter detained in the jugular vein, continuous-drip-infusion impregnation of HGF was carried out at a rate of 100, and a 1000microg/kg / day, and it dissected 72 hours after HGF administration initiation (following administration schedule 3 reference).

[0026]

#### 投与スケジュール 3



[0027] GOT in a blood serum, GPT, total serum protein, and albumin measured the coagulation time of a HEPAPURASUCHIN test with the automatic coagulation ability measuring device (KC-40) about the coagulation ability of the plasma which mixed 3.8% sodium-citrate water solution and the blood extracted from back vena cava at a rate of 1:9 with the Hitachi 7150 mold automatic analyzer. Moreover, the hydroxyproline content (Hyp) in a hepatic tissue was measured by the approach of Kivirikko and others shown above as an index of fibrosis. The result is shown in Table 3 and drawing 2. As shown in Table 3 and drawing 2, the fall of the hydroxyproline content in a hepatic tissue which suggests an improvement of the liver function test value for which HGF depended on the dosage 100microg/kg / above the day, recovery of \*\*\*\*\*, and the improvement of fibrosis was observed.

[0028]

[Table 3]

表 3

	総蛋白質量 (g/dl)	アルブミン量 (g/dl)
溶媒投与群	5.0 ± 0.1	1.8 ± 0.1
HGF投与群(100 μg/kg/日)	5.5 ± 0.2 *	2.1 ± 0.1 *
HGF投与群(1000 μg/kg/日)	5.7 ± 0.2 **	2.1 ± 0.1 *
健常動物	6.0 ± 0.1 **	2.4 ± 0.03 **

平均値 ± 標準偏差 (n = 12 ~ 13)

\* : P < 0.05, \*\* : P < 0.01 溶媒投与群との有意差

[0029] The survival rate in the HGF administration group and the group non-prescribing a medicine for the patient to the liver fibrosis rat caused by the effectiveness \*\* trial ADMN of HGF to the hepatic insufficiency accompanying the rat liver fibrosis and it which were caused by example 3D MN was examined. The administration schedule of drugs is shown above drawing 3. DMN dissolved in the physiological saline by 1% of concentration was injected intraperitoneally at a rate of 10microl on the day shown by the six-week arrow head of 3 time \*\*\* at one week as per

[ DMN ] weight of 1kg of SD system male rat. After DMN administration initiation, from the 21st day, Homo sapiens HGF or a physiological salt solution was administered intravenously every day [ period ] which was shown with the band which carried out hatching, and investigated the survival rate (%) of a trial rat daily. The result is shown in drawing 3 . In drawing 3 , in a dotted line, a physiological-salt-solution administration group (a control group, n= 10) and a broken line show an HGF50microg/kg weight administration group (n= 5), and a continuous line shows an HGF200microg/kg weight administration group (n= 5). As shown in drawing 3 , the survival rate improved by administration of HGF and the example of death was not especially accepted in the HGF200microg/kg weight administration group.

[0030] \*\* The type I collagen deposition (organization view) of rat liver which medicated the bottom of trial BHGF administration or un-prescribing a medicine for the patient with DMN was investigated. That is, for detection of a fibrosis substrate, the rat was killed on the 42nd in the trial shown in drawing 3 , and liver was extracted. Liver was quickly frozen in the OTC compound, and after making the created intercept react with a rabbit origin anti-rat collagen type I antibody (product made from LSL), and a fluorescence labeling goat origin anti-rabbit IgG antibody, it performed photograph shooting of a sample in the immunofluorescent stain of a collagen. The result is shown in drawing 4 . As shown in drawing 4 , there was a significant difference in the deposition of the type I collagen in liver. In the contrast rat of a physiological-salt-solution administration group, the deposition in the liver of a type I collagen is clear, and the east of a thick or thin type I collagen fiber was accumulating it on the blood vessel or the perimeter of hepatocyte extensively. These views disappeared on the dosage dependence target by administration of HGF, and the improvement of leaflet structure was more remarkable in the HGF administration group. Thus, the liver fibrosis prevention effectiveness of HGF became clear from the organization view of a liver intercept.

[0031] \*\* Change of the prothrombin time by trial CHGF administration, a hepatocyte deviation enzyme, and a liver hydroxyproline content was investigated. That is, it dealt with the rat according to the design of experiment shown by drawing 3 . The prothrombin time (PT), albumin (Alb), glutamic oxaloacetic transaminase (GOT), In the value in glutamate pyruvate transaminase (GPT) and the blood serum (plasma) of alkaline phosphatase (ALP), and a list, a liver hydroxyproline content (HYP) A healthy rat group (in n= 5 and Table 4, it is displayed as a healthy rat), the group which carried out DMN processing for five weeks, and prescribed only a physiological salt solution for the patient (in n= 5 and Table 4, it is displayed as 5W), The group which carried out DMN processing for six weeks, and prescribed only a physiological salt solution for the patient (in n= 1 and Table 4) HGF was measured instead of 6W, the display, or a physiological salt solution, respectively by the group prescribed for the patient in 50microg/kg weight (it is displayed as 50 in n= 3 and Table 4), and 200microg/kg weight (it is displayed as 200 in n= 5 and Table 4). The result is shown in Table 4. In addition, front Naka and the value displayed as 5W are values which slaughtered and obtained the rat on the 35th, and values other than this can slaughter a rat after DMN treatment initiation on the 42nd. As shown in Table 4, it became clear by administration of HGF that fibrosis of liver and relaxation of hepatic insufficiency were achieved.

[0032]

[Table 4]

表 4

		PT (sec.)	Alb (mg/dl)	GOT (IU/L)	GPT (IU/L)	ALP (IU/L)	HYP (μg/g Liver)
健康ラット	(n=5)	14.2 ± 2.7	4.6 ± 0.04	76 ± 16	19 ± 3	459.6 ± 65	224 ± 89
生理食塩水	5W (n=5)	—	—	314 ± 71	132 ± 16	1967 ± 236	740 ± 211
	6W (n=1)	80<	2.6	162	49	1268	1011
H G F	50 (n=3)	32.0 ± 11.2	2.6 ± 0.8	165 ± 38	42 ± 14	1081 ± 523	769 ± 58
	200 (n=5)	21.4 ± 5.2	3.1 ± 0.7	164 ± 38	40 ± 12	1053 ± 448	666 ± 116

[0033] To SD system male rat of the 5 weeks old of the liver fiber reduction effectiveness \*\*

approaches by the HGF administration by the example 4DMN liver fibrosis rat, DMN was injected intraperitoneally for four weeks 3 times (the moon, fire, water) per week by the dosage of 10microl/kg, and the liver fibrosis rat was produced to it. This rat was medicated with HGF of 1 mg/kg, or the solvent (HSA 2.5mg/ml, Tween 80 phosphate buffered saline containing 0.01%) of 1 ml/kg from the time of the 5 times (moon, fire, water, tree, gold) DMN administration initiation per week, and a medicine was prescribed for the patient till the 4th for the 4th week. The rat was slaughtered on the 5th for the 4th week, liver was fixed to the neutral formalin after extraction, and the Masson trichrome stain which dyes the fibrous tissue in various colors after intercept production was given. In addition, the liver of a healthy rat was similarly dyed for the comparison. About the liver preparation of the dyed each object, using image-analysis equipment (T.Watanabe et al.Analytical Cellular Pathology 4 (3), 248, 1992), the area of an organization which carried out fibrosis in the whole surface product of an organization intercept was calculated, and extent of fibrosis was measured. In addition, the population used for analysis was six except the individual which HGF neutralization activity generated in the blood serum about eight healthy rats, eight DMN+ solvent administration groups (DMN), and a DMN+HGF administration group (HGF+DMN).

[0034] \*\* The result result was shown in drawing 5 . Each point in drawing shows the rate of the fibrous tissue of the each object liver of a healthy rat (n= 8) and DMN administration rat (n= 8) and a DMN+HGF administration rat (n= 6) at percent. Moreover, the bar of striping shows the average. As shown in drawing 5 , as compared with the healthy rat, the rate of the fibrous tissue under liver tissue increased to 7.3% to an average of 1.3% by DMN. On the other hand, in DMN+HGF, it fell to 2.8% and the liver fibrosis mitigation by DMN was shown by administration of HGF.

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[Translation done.]

\* NOTICES \*

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1. This document has been translated by computer. So the translation may not reflect the original precisely.
2. \*\*\*\* shows the word which can not be translated.
3. In the drawings, any words are not translated.

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DESCRIPTION OF DRAWINGS

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[Brief Description of the Drawings]

[Drawing 1] It is drawing in the trial A of an example 2 showing GOT, GPT, and the measurement result of  $\gamma$ -glutamyl transpeptidase.

[Drawing 2] It is drawing in the trial B of an example 2 showing the measurement result of GOT, GPT, a HEPAPURASUCHIN test, and a liver Hyp (hydroxyproline) content.

[Drawing 3] It is drawing showing the prolongation-of-life effectiveness of HGF to a liver fibrosis rat in the trial A of an example 3. In drawing 3, in a dotted line, a physiological-salt-solution administration group (a control group, n= 10) and a broken line show an HGF50microg/kg weight administration group (n= 5), and a continuous line shows an HGF200microg/kg weight administration group (n= 5).

[Drawing 4] It is the photograph (gestalt of a living thing) in which the liver fibrosis mitigation effectiveness of HGF to a liver fibrosis rat in the trial B of an example 3 is shown.

[Drawing 5] It is drawing of the fibrous tissue of the each object liver in an example 4 showing (%) comparatively.

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[Translation done.]

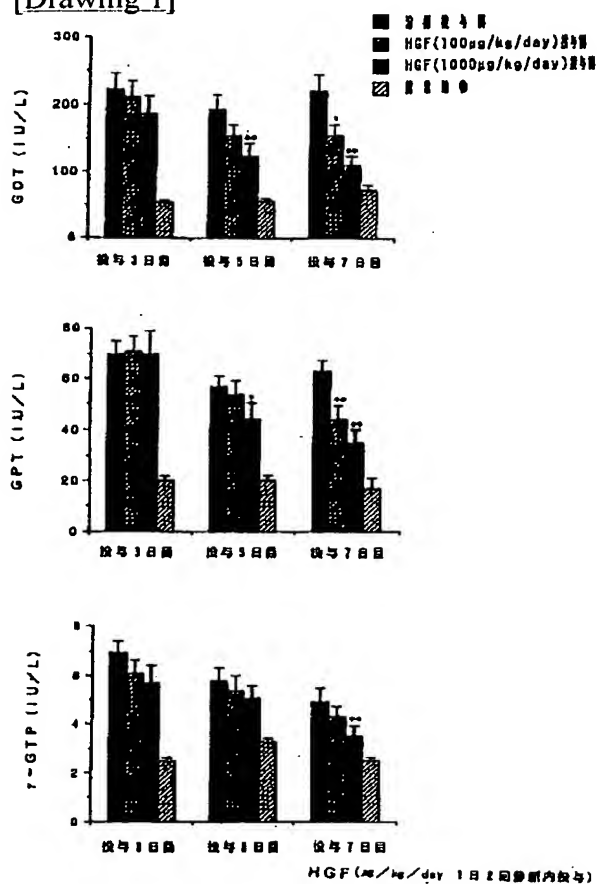
## \* NOTICES \*

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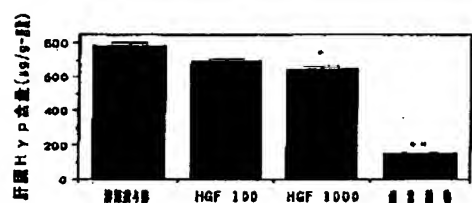
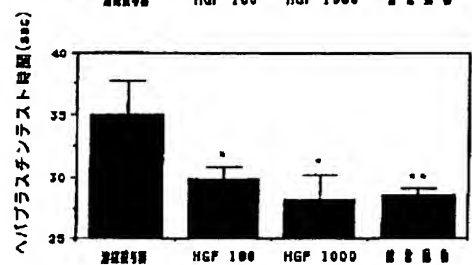
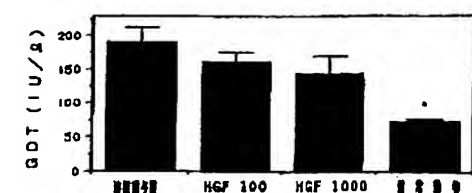
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3. In the drawings, any words are not translated.

## DRAWINGS

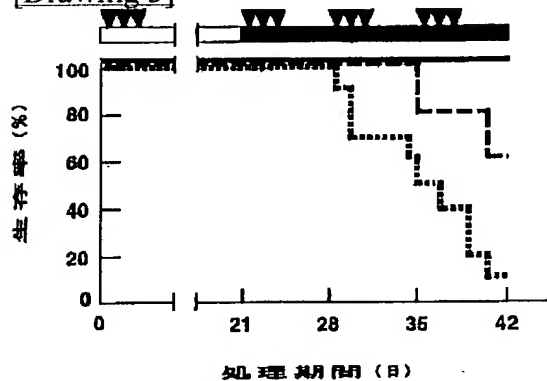
[Drawing 1]



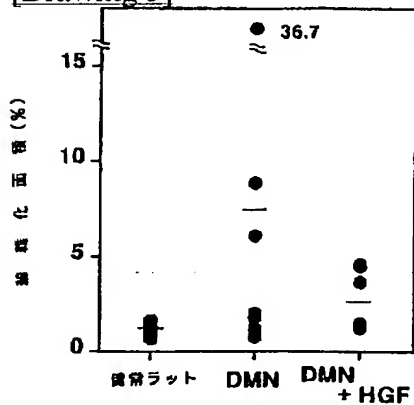
[Drawing 2]



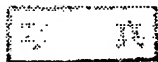
[Drawing 3]



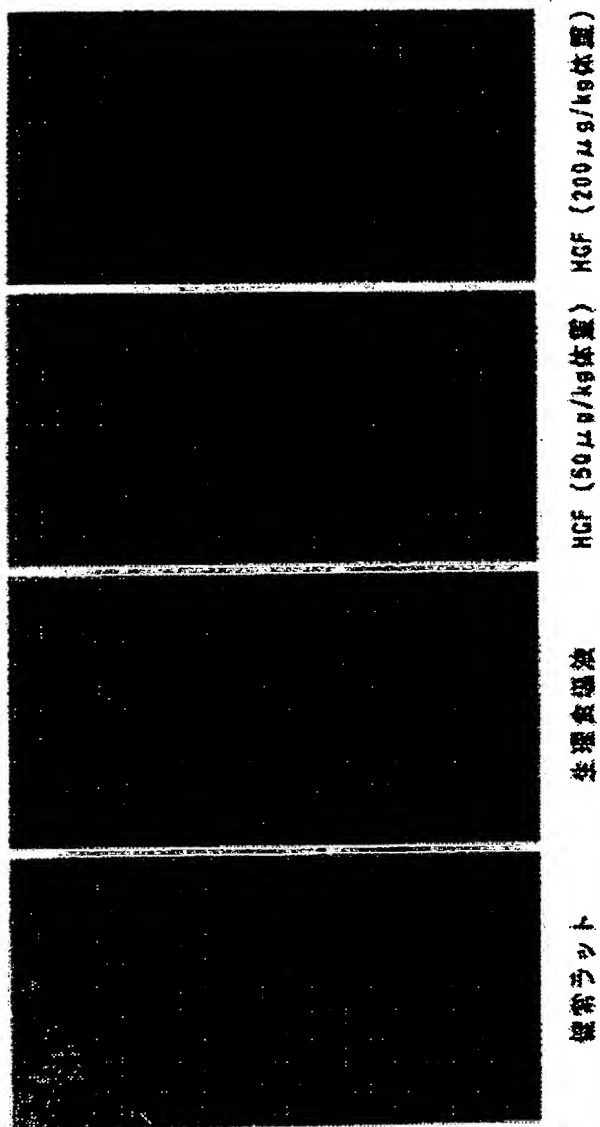
[Drawing 5]



[Drawing 4]



図面代用写真

HGF (200  $\mu$ g/kg 体重)HGF (50  $\mu$ g/kg 体重)

生理食塩液

健常ラット

[Translation done.]

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	ABJ			
	ACD			

A 6 1 K 37/ 24

ABX

ABJ

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(54) 【発明の名称】 コラーゲン分解促進剤

(57) 【要約】

【目的】 線維症障害の治療に有用なコラーゲン分解促進剤及び線維症障害治療剤を提供することを目的とする。

【構成】 本発明のコラーゲン分解促進剤及び線維症障害治療剤は、それぞれHGF (Hepatocyte Growth Factor, 肝細胞増殖因子) 類を有効成分として含有することからなる。有効成分であるHGF類は、コラーゲンの分解(コラゲナーゼ活性の増加)を促進する作用を有しており、線維症障害を有効に治療することができる。



## 【特許請求の範囲】

【請求項1】 HGF類の少なくとも一種を有効成分として含有することを特徴とするコラーゲン分解促進剤。

【請求項2】 HGF類として、HGFを含有する請求項1記載のコラーゲン分解促進剤。

【請求項3】 HGF類の少なくとも一種を有効成分として含有することを特徴とする線維症障害治療剤。

【請求項4】 上記線維症障害が、動脈硬化、慢性糸球体腎炎、皮膚ケロイド生成、進行性全身性硬化症(PSS)、肝線維症、肺線維症、嚢胞性線維症、慢性の対宿主移植片疾患、硬皮症(局部及び全身)、ペイロニー氏病、陰茎の線維症、膀胱鏡検査後の尿道狭窄症、外科手術後の内部癒着、骨髄線維症及び特発性後部腹膜線維症からなる群から選ばれることを特徴とする請求項3記載の線維症障害治療剤。

【請求項5】 HGF類として、HGFを含有する請求項3又は4記載の線維症障害治療剤。

## 【発明の詳細な説明】

## 【0001】

【産業上の利用分野】本発明は、コラーゲン分解促進剤及び線維症障害治療剤に関する。より詳細には、HGF(Hepatocyte Growth Factor)類を有効成分としたコラーゲン分解促進剤及び線維症障害治療剤に関する。

## 【0002】

【従来の技術】線維症とは、結合組織成分の過度の蓄積で特徴づけられる疾患であり、線維症においてその中心をなし最も注目すべきものはコラーゲンである。コラーゲンの蓄積は種々の内臓で生じ、例えば、肺においては肺線維症、肝臓においては肝線維症のような病態で生じる。また、皮膚においても、例えば、皮膚ケロイド生成のような病態で生じる。多くの場合、線維症におけるコラーゲンの正味の蓄積は、コラーゲンの生産及び分解を起す因子の間の不均衡の結果である。線維症の疾患及び障害を治療するために種々の投薬法が行われてきたが、一般にそれらは障害の対症療法が中心であり、そのもとである病理すなわちコラーゲンその他の結合組織成分の生産及び分解を調節する代謝因子のなかの不均衡を解消することを目指したものではなかった。それゆえ、これらの治療法のなかには、組織の改善という点で特に有効とされるものはなかった。すなわち、例えば、皮膚ケロイド生成の初期の炎症段階を治療するのに局部コルチコステロイドが使用されて或る程度成功したが、過度のコラーゲン生産の結果としてケロイドが実際に生成した場合のような後期の線維症の段階ではそのようなステロイドは殆ど又は全く効果がない。このように、従来技術では、安全で有効な方法でヒトの線維症障害を治療して、それ以上の線維症の組織の生成を阻止し、既に生成している線維症の病巣を減少させ又は完全に除去するような方法は見出せなかった。

## 【0003】

【発明が解決しようとする課題】本発明が解決しようとする課題は、組織内における過度に蓄積された結合性組織のコラーゲンマトリックスの分解を誘導することのできるコラーゲン分解促進剤及び線維症障害の治療に有用な治療剤を提供することにある。

## 【0004】

【課題を解決するための手段】本発明者等は、上記の課題を解決するために鋭意検討を重ねた結果、HGF類がコラーゲンの分解を促進する作用を有し、その作用に基づき線維症障害の治療に有効であることを見出し、本発明を完成させた。すなわち、本発明は、

①HGF類の少なくとも一種を有効成分として含有することを特徴とするコラーゲン分解促進剤；

②HGF類の少なくとも一種を有効成分として含有することを特徴とする線維症障害治療剤；に関する。

【0005】本発明において、HGF類とは、肝細胞増殖活性を示す蛋白質をいい、例えば、HGF(Hepatocyte Growth Factor)などが挙げられる。本発明で使用されるHGF類としては、医薬として使用できる程度に精製されたものであれば、種々の方法で調製されたものを用いることができる。HGFの調製方法としては、各種の方法が知られている。例えば、ラット、ウシ、ウマ、ヒツジなどの哺乳動物の肝臓、脾臓、肺臓、骨髓、脳、腎臓、胎盤等の臓器、血小板、白血球等の血液細胞や血漿、血清などから抽出、精製して得ることができる(FEB S Letters, 224, 312, 1987, Proc. Natl. Acad. Sci. USA, 86, 5844, 1989など参照)。また、HGFを産生する初代培養細胞や株化細胞を培養し、培養物(培養上清、培養細胞等)から分離精製してHGFを得ることもできる。あるいは遺伝子工学的手法によりHGFをコードする遺伝子を適切なベクターに組み込み、これを適当な宿主に挿入して形質転換し、この形質転換体の培養上清から目的とする組換えHGFを得ることができる(例えば、Nature, 342, 440, 1989, Biochem. Biophys. Res. Commun., 163, 967, 1989など参照)。上記の宿主細胞は特に限定されず、従来から遺伝子工学的手法で用いられている各種の宿主細胞、例えば大腸菌、枯草菌、酵母、糸状菌、植物又は動物細胞などを用いることができる。

【0006】より具体的には、HGFを生体組織から抽出精製する方法としては、例えば、ラットに四塩化炭素を腹腔内投与し、肝炎状態にしたラットの肝臓を摘出して粉碎し、S-セファロース、ヘパリンセファロースなどのゲルカラムクロマトグラフィー、HPLC等の通常の蛋白質精製法にて精製することができる。また、遺伝子組換え法を用い、ヒトHGFのアミノ酸配列をコードする遺伝子を、ウシバビローマウィルスDNAなどのベクターに組み込んだ発現ベクターによって動物細胞、例えば、チャイニーズハムスター卵巣(CHO)細胞、マ

ウスC127細胞、サルCOS細胞などを形質転換し、その培養上清より得ることができる。

【0007】かくして得られたHGF類は、HGF類と実質的に同効である限り、そのアミノ酸配列の一部が欠失又は他のアミノ酸により置換されていたり、他のアミノ酸配列が一部挿入されていたり、N末端及び／又はC末端に1又は2以上のアミノ酸が結合していたり、あるいは糖鎖が同様に欠失又は置換されていたりしてもよい。

【0008】本発明のコラーゲン分解促進剤は上記のHGF類を有効成分とし、HGF類は後記試験例に示されるように、コラーゲンの分解（コラーゲナーゼ活性の増加）を促進する作用を有する。従って、下記の線維症障害の治療の他、その予防にも有用であり、またコラーゲナーゼ活性が低下した疾患、例えば、大理石骨病などの治療・予防に有用である。また、本発明の線維症障害治療剤は、同様に上記のHGF類を有効成分とし、コラーゲン、フィブロネクチン及びグリコサミノグリカン（GAG）を含む結合性組織マトリックスの過度の線維芽細胞生産によって特徴づけられる線維症障害の治療に有用である。これには下記の障害が含まれる。

【0009】動脈硬化、慢性糸球体腎炎、皮膚ケロイド生成、進行性全身性硬化症（PSS）、肝線維症、肺線維症、嚢胞性線維症、慢性の対宿主移植片疾患、硬皮症（局部及び全身）、ペイロニー氏病、陰茎の線維症、膀胱鏡検査後の尿道狭窄症、外科手術後の内部癒着、骨髄線維症、特発性後部腹膜線維症

【0010】本発明のコラーゲン分解促進剤及び線維症障害治療剤は、ヒトの他、哺乳動物（例えば、ウシ、ウマ、ブタ、ヒツジ、イヌ、ネコ等）におけるコラーゲン分解促進及び線維症障害治療に適用される。

【0011】本発明のコラーゲン分解促進剤及び線維症障害治療剤は種々の製剤形態（例えば、液剤、固形剤、カプセル剤等）をとりうるが、一般的には有効成分であるHGF類のみ又はそれと慣用の担体と共に注射剤、吸入剤、坐剤又は経口剤とされる。当該注射剤は常法により調製することができ、例えば、HGF類を適切な溶剤（例えば、滅菌された水、緩衝液、生理食塩水等）に溶解した後、フィルター等で濾過して滅菌し、次いで無菌的な容器に充填することにより調製することができる。注射剤中のHGF類含量としては、通常0.0002～0.2（W/V%）程度、好ましくは0.001～0.1（W/V%）程度に調整される。また、経口薬としては、例えば、錠剤、顆粒剤、細粒剤、散剤、軟又は硬カプセル剤、液剤、乳剤、懸濁剤、シロップ剤などの剤形に製剤化され、これらの製剤は製剤化の常法に準じて調製することができる。坐剤も慣用の基剤（例えば、カカオ脂、ラウリン脂、グリセロールゼラチン、マクロゴール、ウィテップゾル等）を用いた製剤上の常法によって調製することができる。また、吸入剤も製剤上の常法に準じて調製することができる。製剤中のHGF類含量は、剤形、適用疾患などに応

じて適宜調整することができる。

【0012】製剤化に際して、好ましくは安定化剤が添加され、安定化剤としては、例えば、アルブミン、グロブリン、ゼラチン、グリシン、マンニトール、グルコース、デキストラン、ソルビトール、エチレングリコールなどが挙げられる。さらに、本発明の製剤は製剤化に必要な添加物、例えば、賦形剤、溶解補助剤、酸化防止剤、無痛化剤、等張化剤等を含んでいてもよい。液状製剤とした場合は凍結保存、又は凍結乾燥等により水分を除去して保存するのが望ましい。凍結乾燥製剤は、用時に注射用蒸留水などを加え、再溶解して使用される。

【0013】本発明のコラーゲン分解促進剤及び線維症障害治療剤は、その製剤形態に応じた適当な投与経路により投与され得る。例えば、注射剤の形態にして静脈、動脈、皮下、筋肉内などに投与することができる。その投与量は、患者の症状、年齢、体重などにより適宜調整されるが、通常HGF類として0.05mg～500mg、好ましくは1mg～100mgであり、これを1日1回ないし数回に分けて投与するのが適当である。

【0014】

【発明の効果】本発明の有効成分であるHGF類は、コラーゲンの分解（コラーゲナーゼ活性の増加）を促進し、もって線維症障害を有効に治療することができる。

【0015】

【実施例】以下、製造例及び実施例に基づいて本発明をより詳細に説明するが、本発明はこれらの例に限定されるものではない。

#### 製造例1

##### HGF製剤の生産例

- 30 (1) HGF 20  $\mu$ g  
ヒト血清アルブミン 100 mg  
上記物質をpH7.0の0.01MのPBSで溶解し、全量を20mlに調製し、滅菌後、バイアル瓶に2mlずつ分注し、凍結乾燥密封した。
- (2) HGF 40  $\mu$ g  
ツイーン80 1 mg  
ヒト血清アルブミン 100 mg  
上記物質を注射用生理食塩水に溶解し、全量を20mlに調製し、滅菌後、バイアル瓶に2mlずつ分注し、凍結乾燥密封した。

#### 【0016】実施例1

##### ジメチルニトロサミン肝線維化ラットに対するHGFの線維化抑制作用と症状改善効果

##### 1. 試験方法

①使用動物：ウィスター系雄ラット、5週齢

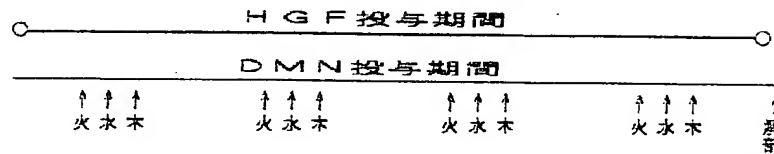
②試験スケジュール

ジメチルニトロサミン（DMN）を毎週火、水、木曜日に10  $\mu$ l/kgの用量で4週にわたり腹腔内投与した。HGFはDMN初回投与時より500  $\mu$ g/kgを1日2回（1000  $\mu$ g/kg/日）28日間静脈内投

与した(下記投与スケジュール1参照)。29日目に試験 \* {0017}

ラットを下記の測定に供した。 \*

### 投与スケジュール1



### 【0018】③測定

ラットを解剖して肝重量を測定した。また、肝組織中のヒドロキシプロリン含量(Hyp;線維化の指標)及びコラゲナーゼ(コラーゲン分解酵素)活性を、それぞれKivirikkoらの方法(Anal. Biochem, 19, 249, 1967)及びMurawakiらの方法(J. Biochem, 108, 241, 1990)により測定した。更に、肝組織中のDNA及び蛋白含量は、それぞれDishe法のBurtonによる変法(Biochem, J, 62, 315, 1956)及びプロテインアッセイキット(バイオラット社製)により測定した。その結果を表1に示す。また、同時に後大静脈より採血し、採取した血清の臨床生化学検査は日立7150型自動分析装置により分析した。血液検査は、※20

表1

	溶媒投与群	HGF投与群	健常動物
肝重量(g)	9.33±0.72	13.02±0.53 **	13.59±0.51 **
総DNA量(μg/肝臓)	33.6±2.5	39.3±1.8 *	44.8±1.7 **
総蛋白量(g/肝臓)	1.36±0.12	1.34±0.09 **	2.33±0.09 **
コラゲナーゼ活性 (μg/min/g-肝臓)	0.22±0.01	0.36±0.07 **	0.27±0.02
ヒドロキシプロリン含量 (μg/g-肝臓)	423.1±35.9	300.1±18.0 **	129.3±6.4 **

平均値±標準誤差 (n=10)

\*: P<0.05, \*\*: P<0.01 溶媒投与群との有意差

【0020】

【表2】

※後大静脈より採取したEDTA加血液で血小板数、白血球数、赤血球数、ヘマトクリット値、ヘモグロビン濃度を多項目自動血球計数装置(E-4000, Sysmex)を用いて測定した。また、3.8%クエン酸ナトリウム水溶液と後大静脈より採取した血液を1:9の割合で混合した血漿で、血漿凝固能(プロトロンビン時間、フィブリノーゲン量、ヘパラスチンテストとトロンボテストによる凝固時間)を自動凝固能測定装置(KC-40)を用いて測定した。その結果を表2に示す。

【0019】

【表1】

表2

血液生化学検査値	溶媒投与群	HGF投与群	健常動物
GOT (IU/l)	136±9	78±4 **	64±3 **
GPT (IU/l)	50±4	28±1 **	15±1 **
γ-GTP (IU/l)	4.8±0.4	3.4±0.2 **	1.9±0.1 **
総ビリルビン (mg/dl)	0.45±0.08	0.25±0.01 **	0.19±0.01 **
直接型ビリルビン (mg/dl)	0.20±0.02	0.18±0.00	0.13±0.01 **
総蛋白 (g/dl)	4.9±0.1	6.4±0.1 **	5.7±0.0 **
アルブミン (g/dl)	2.4±0.1	3.1±0.1 **	2.6±0.0 **
血糖値 (mg/dl)	131±4	152±6 **	180±8 **
総コレステロール (mg/dl)	53±2	98±5 **	72±3 **
HDL-コレステロール (mg/dl)	28.0±1.9	61.6±3.9 **	41.3±1.7 **
トリグリセリド (mg/dl)	70±9	152±16 **	157±16 **
リン脂質 (mg/dl)	118±4	208±8 **	160±5 **
βリポ蛋白 (mg/dl)	107±11	222±20 **	202±18 **
血液・凝固検査値	溶媒投与群	HGF投与群	健常動物
血小板数 (10 <sup>4</sup> /μl)	31±5	78±4 **	105±5 **
白血球数 (10 <sup>3</sup> /μl)	144±6	101±8 **	87±3 **
赤血球数 (10 <sup>6</sup> /μl)	667±16	702±9 **	755±6 **
ヘマトクリット値 (%)	39.9±0.9	41.6±0.3 *	44.3±0.3 **
ヘモグロビン濃度 (g/dl)	12.7±0.3	13.6±0.1 **	14.6±0.1 **
プロトロンビン時間 (sec)	15.6±0.5	13.8±0.2 *	13.8±0.2 *
フィブリノーゲン (g/dl)	1.45±0.10	2.05±0.11 **	2.31±0.05 **
ヘパプラスチン時間 (sec)	37.3±2.7	28.6±0.6 **	28.5±0.5 **
トロンボテスト時間 (sec)	30.0±1.9	22.5±0.3 **	22.8±0.3 **

平均値±標準誤差 (n=10)

\*: P&lt;0.05, \*\*: P&lt;0.01 溶媒投与群との有意差

## 【0021】2. 結果

表1及び表2に示すように、DMNの反復投与により、溶媒投与群（対照）では顕著な肝の線維化の伸展と萎縮が観察され、臨床生化学、血液及び凝固系検査値から明らかな肝機能の低下が認められた。これに対し、HGF投与群の肝機能検査値は溶媒投与群のそれらの値より有意な差で健常動物に近い値を示し、明らかな改善効果を示していた。また、HGF投与により肝組織中のDNA、蛋白量、コラゲナーゼ活性が有意に上昇し、線維化の指標であるヒドロキシプロリン含量は有意に低下し、肝重量はほぼ正常レベルまで回復した。

## 【0022】実施例2

## 四塩化炭素肝線維化ラットに対するHGFの作用

ウィスター系雄ラット（6週齢）に四塩化炭素を毎週月、木曜日に0.7ml/kgの用量で12週間経口投

与して肝線維化モデルを作成した。この四塩化炭素肝線維化ラットを下記の2つの試験に供した。

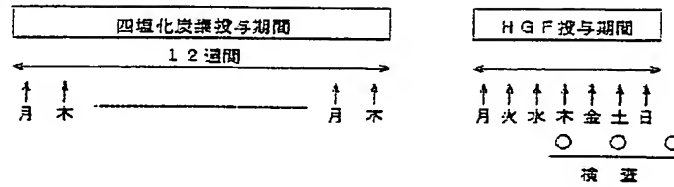
## 【0023】①試験A

## 反復投与試験

上記の四塩化炭素肝線維化ラット（1群13～14匹）に、HGFを50及び500μg/kgで1日2回（100及び1000μg/kg/日）7日間静脈内投与した。HGF投与開始から3、5、7日目の血清中のGOT、GPT、γ-GTP値の変化を溶媒投与群（対照）と比較した（下記投与スケジュール2参照）。その結果を図1に示す。図1に示されるように、1000μg/kg/日で5日目、100μg/kg/日で7日目から回復促進作用が認められた。

## 【0024】

9  
投与スケジュール2



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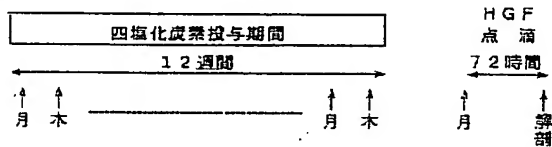
## 【0025】②試験B

## 点滴注入試験

各群12～13匹の上記四塩化炭素肝線維化ラットを用い、頸静脈に留置したカテーテルより、HGFを100及び1000 $\mu$ g/kg/日の割合で持続点滴注入し、HGF投与開始から72時間後に解剖した(下記投与スケジュール3参照)。

## 【0026】

## 投与スケジュール3



\*【0027】血清中のGOT、GPT、血清総蛋白、アルブミンは日立7150型自動分析装置により、3.8%クエン酸ナトリウム水溶液と後大静脈より採取した血液を1:9の割合で混合した血漿の凝固能については、ヘパラスチンテストの凝固時間を自動凝固能測定装置(KC-40)で測定した。また、線維化の指標として肝組織中ヒドロキシプロリン含量(Hyp)は、前掲のKivirikkoらの方法により測定した。その結果を表3及び図2に示す。表3及び図2に示されるように、HGFは100 $\mu$ g/kg/日以上で用量に依存した肝機能検査値の改善、低蛋白血症の回復及び線維化の改善を示唆する肝組織中ヒドロキシプロリン含量の低下が観察された。

## 【0028】

## 【表3】

20  
\*  
表3

	総蛋白量 (g/dl)	アルブミン量 (g/dl)
溶媒投与群	5.0 $\pm$ 0.1	1.8 $\pm$ 0.1
HGF投与群(100 $\mu$ g/kg/日)	5.5 $\pm$ 0.2 *	2.1 $\pm$ 0.1 *
HGF投与群(1000 $\mu$ g/kg/日)	5.7 $\pm$ 0.2 **	2.1 $\pm$ 0.1 *
健常動物	6.0 $\pm$ 0.1 **	2.4 $\pm$ 0.03 **

平均値 $\pm$ 標準偏差 (n=12～13)

\*: P<0.05, \*\*: P<0.01 溶媒投与群との有意差

## 【0029】実施例3

## DMNによって惹起されたラット肝線維化とそれに伴う肝機能不全に対するHGFの効果

## ①試験A

DMNによって惹起された肝線維化ラットに対するHGF投与群及び非投与群における生存率を試験した。薬剤の投与スケジュールを図3の上方に示す。生理食塩水に1%の濃度で溶解したDMNを、SD系雄性ラットの体重1kgあたりDMNとして10 $\mu$ lの割合で、1週間に3回つづ6週間矢印で示した日に腹腔内投与した。ヒトHGF又は生理食塩液は、DMN投与開始後21日目よりハッチングした帯で示した期間毎日静脈内投与し、試験ラットの生存率(%)を経日的に調べた。その結果を図3に示す。図3において、点線は生理食塩液投与群(対照群、n=10)、破線はHGF50 $\mu$ g/kg体重投与群(n=5)、実線はHGF200 $\mu$ g/kg体重投与群(n=5)を示す。図3に示されるように、HGFの投与により生存率が向上し、特にHGF200 $\mu$ g/kg体重投与群においては死亡例は認められなかつ

た。

## 【0030】②試験B

HGF投与又は非投与下において、DMNを投与したラット肝臓のタイプIコラーゲン沈着(組織所見)を調べた。即ち、線維化基質の検出のために、図3に示した試験において42日目にラットを殺し肝臓を採取した。コラーゲンの免疫蛍光染色には、肝臓をOTC化合物のなかですばやく凍結し、作成した切片はウサギ由来抗ラットコラーゲンタイプI抗体(LSL社製)、そして蛍光標識化ヤギ由来抗ウサギIgG抗体と反応させた後、標本の写真撮影を行った。その結果を図4に示す。図4に示されるように、肝臓におけるタイプIコラーゲンの沈着には有意な違いがあった。生理食塩液投与群の対照ラットでは、タイプIコラーゲンの肝臓での沈着は明瞭であり、血管や肝細胞周囲に太い或いは細いタイプIコラーゲン線維の束が広範に集積していた。これらの所見はHGFの投与によって用量依存的に消失し、また小葉構造の改善はHGF投与群においてより顕著であった。このように、HGFの肝線維化防止効果は肝臓切片の組織

所見から明らかになった。

#### 【0031】③試験C

HGF投与による、プロトロンビン時間、肝細胞逸脱酵素及び肝ヒドロキシプロリン含量の変化を調べた。即ち、ラットは図3で示した実験計画に従い処置した。プロトロンビン時間(PT)、アルブミン(Alb)、グルタミン酸オキサロ酢酸トランスアミナーゼ(GOT)、グルタミン酸ピルビン酸トランスアミナーゼ(GPT)及びアルカリフォスファターゼ(ALP)の血清(血漿)中の値、並びに肝ヒドロキシプロリン含量(HYP)は、健常ラット群(n=5、表4において健常ラットと表示)、DMN処理を5週間し生理食塩液のみを投与した群(n=5、表4において5Wと表示)、DM\*

表4

		PT (sec.)	Alb (mg/dl)	GOT (IU/L)	GPT (IU/L)	ALP (IU/L)	HYP ( $\mu$ g/g Liver)
健常ラット	(n=5)	14.2 $\pm$ 2.7	4.5 $\pm$ 0.04	78 $\pm$ 16	19 $\pm$ 3	459.6 $\pm$ 85	224 $\pm$ 89
生理食塩液	5W (n=5)	—	—	314 $\pm$ 71	132 $\pm$ 16	1967 $\pm$ 295	740 $\pm$ 211
	5W (n=1)	80<	2.5	162	49	1268	1011
H G F	50 (n=3)	32.0 $\pm$ 11.2	2.5 $\pm$ 0.3	165 $\pm$ 38	42 $\pm$ 14	1081 $\pm$ 523	789 $\pm$ 58
	200 (n=5)	21.4 $\pm$ 5.2	3.1 $\pm$ 0.7	164 $\pm$ 38	40 $\pm$ 12	1053 $\pm$ 448	668 $\pm$ 116

#### 【0033】実施例4

##### DMN肝線維化ラットでのHGF投与による肝線維減少効果

##### ①方法

5週齢のSD系雄性ラットに、DMNを10  $\mu$ l/kgの用量で週3回(月、火、水)4週間腹腔内投与し肝線維化ラットを作製した。このラットに1mg/kgのHGF又は1ml/kgの溶媒(HSA 2.5mg/ml、ツイーン80 0.01%を含むリン酸緩衝生理食塩水)を週5回(月、火、水、木、金)DMN投与開始時より投与し、4週目の4日目まで投与した。4週目の5日目にラットを屠殺、肝臓を採取後中性ホルマリンに固定し、切片作製後に線維組織を染め分けるマッソントリクローム染色を施した。なお、比較のために健常ラットの肝臓も同様に染色した。染色した各個体の肝臓組織標本について、画像解析装置(T. Watanabe et al. Analytical Cellular Pathology 4 (3), 248, 1992)を用い、組織切片の全面積中の線維化した組織の面積を計算して線維化の程度を比較した。なお、解析に用いた個体数は、健常ラット8例、DMN+溶媒投与群(DMN)8例、DMN+HGF投与群(HGF+DMN)については血清中にHGF中和活性の生成した個体を除いた6例であった。

##### 【0034】②結果

結果を図5に示した。図中の各点は、健常ラット(n=

\*N処理を6週間し生理食塩液のみを投与した群(n=1、表4において6Wと表示)又は生理食塩液の代りにHGFを50  $\mu$ g/kg体重(n=3、表4において50と表示)と200  $\mu$ g/kg体重(n=5、表4において200と表示)で投与した群でそれぞれ測定した。その結果を表4に示す。なお、表中、5Wと表示した値は35日目にラットを屠殺して得た値であり、これ以外の値はDMN処置開始後42日目にラットを屠殺し得たものである。表4に示されるように、HGFの投与により、肝臓の線維化及び肝機能不全の緩和が図られていることが判明した。

#### 【0032】

【表4】

8)、DMN投与ラット(n=8)、DMN+HGF投与ラット(n=6)の各個体肝臓の線維組織の割合をパーセントで示したものである。また、横線のバーは平均値を示す。図5に示されるように、健常ラットと比較すると、DMNでは肝臓組織中の線維組織の割合が、平均1.3%に対して7.3%に増加した。これに対してDMN+HGFでは2.8%に低下し、HGFの投与によってDMNによる肝線維化軽減が示された。

##### 【図面の簡単な説明】

【図1】実施例2の試験Aにおける、GOT、GPT及び $\gamma$ -GTPの測定結果を示す図である。

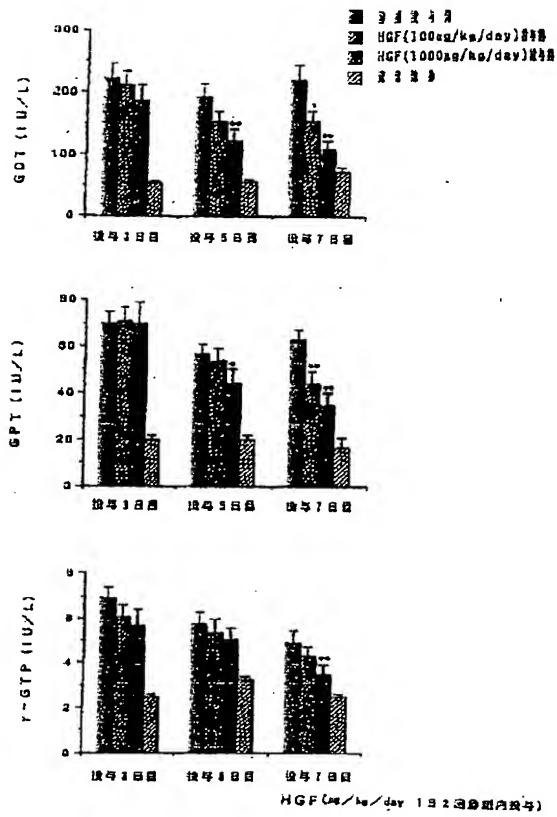
【図2】実施例2の試験Bにおける、GOT、GPT、ヘパラスチンテスト及び肝臓Hyp(ヒドロキシプロリン)含量の測定結果を示す図である。

【図3】実施例3の試験Aにおける、肝線維化ラットに対するHGFの延命効果を示す図である。図3において、点線は生理食塩液投与群(対照群、n=10)、破線はHGF50  $\mu$ g/kg体重投与群(n=5)、実線はHGF200  $\mu$ g/kg体重投与群(n=5)を示す。

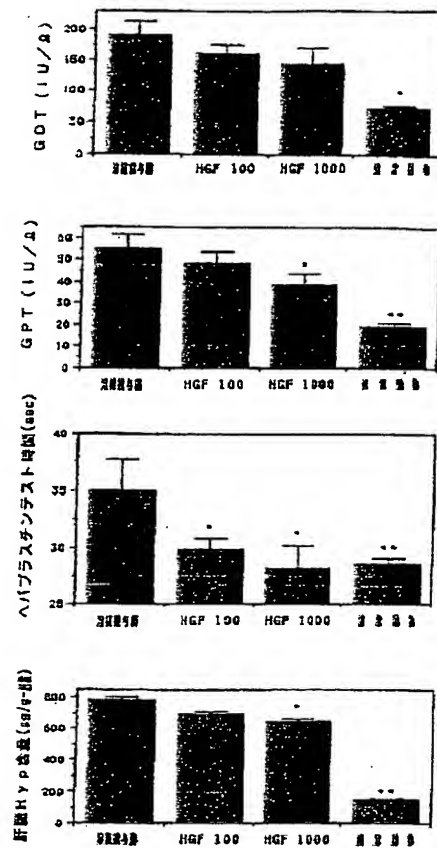
【図4】実施例3の試験Bにおける、肝線維化ラットに対するHGFの肝線維化軽減効果を示す写真(生物の形態)である。

【図5】実施例4における、各個体肝臓の線維組織の割合(%)を示す図である。

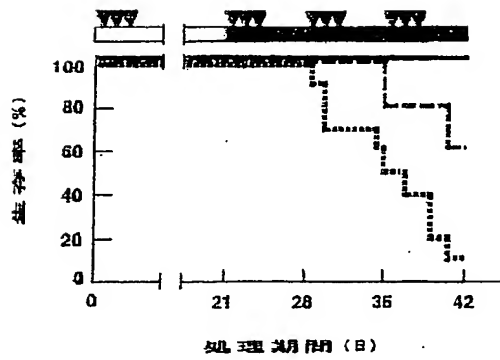
【図1】



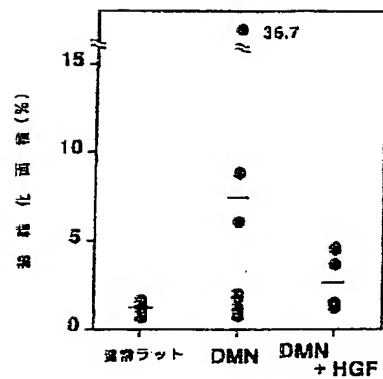
【図2】



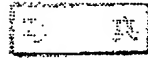
【図3】



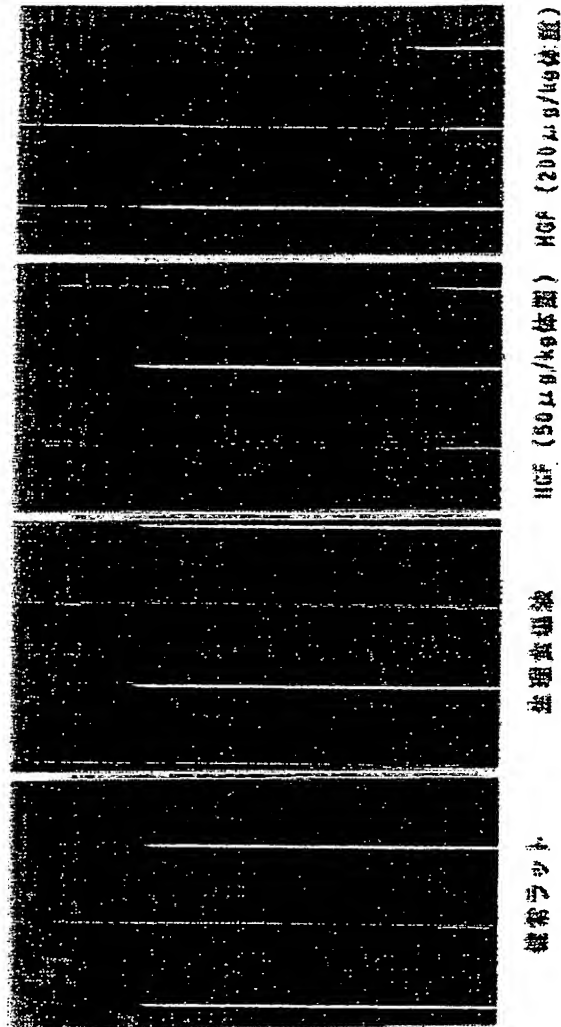
【図5】



【図4】



図面代用写真



フロントページの続き

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CLAIMS

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[Claim(s)]

[Claim 1] The collagenolysis accelerator characterized by the thing of HGF for which a kind is contained as an active principle at least.

[Claim 2] The collagenolysis accelerator according to claim 1 which contains HGF as HGF.

[Claim 3] The fibrosing disease failure therapy agent characterized by the thing of HGF for which a kind is contained as an active principle at least.

[Claim 4] The fibrosing disease failure therapy agent according to claim 3 characterized by being chosen out of the group which the above-mentioned fibrosing disease failure becomes from arteriosclerosis, the chronic glomerulonephritis, skin keloid generation, progressive systemic sclerosis (PSS), hepatic fibrosis, the fibroid lung, the cystic fibrosis, the chronic transplant disease for a host, the scleroderma (a part and whole body), a pay RONI Mr. disease, the fibrosing disease of a phallus, the urethrostenosis after the cystoscopy, the internal adhesion after a surgical operation, the myelofibrosis, and an idiopathic posterior part peritoneum fibrosing disease.

[Claim 5] The fibrosing disease failure therapy agent according to claim 3 or 4 which contains HGF as HGF.

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[Translation done.]

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DETAILED DESCRIPTION

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[Detailed Description of the Invention]

[0001]

[Industrial Application] This invention relates to a collagenolysis accelerator and a fibrosing disease failure therapy agent. It is related more with a detail at the collagenolysis accelerator and fibrosing disease failure therapy agent which made HGF (Hepatocyte Growth Factor) the active principle.

[0002]

[Description of the Prior Art] A fibrosing disease is a disease characterized by too much are recording of a connective tissue component, and it is the collagen which the core should be made in a fibrosing disease and should be observed most. It is generated in various internal organs, for example, are recording of a collagen is produced in symptoms like hepatic fibrosis in the fibroid lung and liver in lungs. Moreover, also in the skin, it is generated in symptoms like skin keloid generation, for example. In many cases, are recording of the net of the collagen in a fibrosing disease is a result out of balance between the factors which cause production and decomposition of a collagen. Although various medication had been performed in order to treat the disease and failure of a fibrosing disease, generally they were not what aimed at that the symptomatic therapy of a failure canceled the imbalance in the metabolic turnover factor which adjusts production and decomposition of the pathology, i.e., a collagen and others, which is a core and is the basis of a connective tissue component. So, there was especially nothing that is confirmed in respect of an improvement of an organization into these cures. namely, -- for example, local corticosteroid uses it for treating the inflammation phase in early stages of skin keloid generation -- having -- such [ in the phase of the fibrosing disease of an anaphase like / when / a certain / keloid actually generates as a result of too much collagen production / although an extent success was carried out ] a steroid -- most -- or it is completely ineffective. Thus, with the conventional technique, an approach which a human fibrosing disease failure is treated by the safe and effective approach, generation of the organization of the fibrosing disease beyond it is prevented, and the focus of the fibrosing disease already generated is decreased, or is removed completely was not able to be found out.

[0003]

[Problem(s) to be Solved by the Invention] The technical problem which this invention tends to solve is to offer the collagenolysis accelerator which can guide decomposition of the collagen matrix of an affinity organization in an in-house accumulated too much, and a therapy agent useful for the therapy of a fibrosing disease failure.

[0004]

[Means for Solving the Problem] In order to solve the above-mentioned technical problem, as a result of repeating examination wholeheartedly, this invention person etc. has the operation to which HGF promotes decomposition of a collagen, and completed a header and this invention for it being effective in the therapy of a fibrosing disease failure based on the operation. That is, this invention is a collagenolysis accelerator characterized by the thing of \*\*HGF for which a kind is contained as an active principle at least.;

\*\* It is related with fibrosing disease failure therapy agent; characterized by the thing of HGF for which a kind is contained as an active principle at least.

[0005] In this invention, HGF says the protein in which hepatocyte growth activity is shown, for example, HGF (Hepatocyte Growth Factor) etc. is mentioned. If refined as HGF used by this

invention by extent which can be used as physic, what was prepared by various approaches can be used. Various kinds of approaches are learned as the preparation approach of HGF. For example, it can extract and refine and can obtain from blood cells, such as organs, such as the liver of mammals, such as a rat, a cow, a horse, and a sheep, a spleen, a lung, bone marrow, a brain, the kidney, and a placenta, a platelet, and a leucocyte, plasma, a blood serum, etc. (reference, such as FEBS Letters, 224, 312, 1987, Proc.Natl.Acad.Sci.USA, 86, 5844, and 1989). Moreover, the primary culture cell and established cell line which produce HGF can be cultivated, separation purification can be carried out from cultures (a culture supernatant, cultured cell, etc.), and HGF can also be obtained. Or recombination HGF which inserts in a suitable vector the gene which carries out the code of HGF by the gene engineering-technique, inserts a nest and this in a suitable host, and carries out a transformation and which is made into the purpose from the culture supernatant of this transformant can be obtained (for example, reference, such as Nature, 342, 440, 1989, Biochem.Biophys.Res.Comm., 163, 967, and 1989). Especially the above-mentioned host cell is not limited, but can use various kinds of host cells used by the gene engineering-technique from the former, for example, Escherichia coli, a Bacillus subtilis, yeast, mold, vegetation, or an animal cell. [0006] As an approach of carrying out extract purification of HGF from a body tissue, a carbon tetrachloride can be injected intraperitoneally to a rat, the liver of the rat changed into the hepatitis condition can be extracted and ground, and, more specifically, it can refine in the usual protein purification methods, such as gel column chromatographies, such as S-sepharose and heparin sepharose, and HPLC, for example. Moreover, using the modifying-gene method, by the expression vector which included the gene which carries out the code of Homo sapiens's HGF amino acid sequence in vectors, such as a bovine papilloma virus DNA, the transformation of an animal cell, for example, a Chinese hamster ovary cell (CHO) cell, mouse C127 cell, the ape COS cell, etc. can be carried out, and it can obtain from the culture supernatant.

[0007] as long as HGF obtained in this way is these effects as substantially as HGF -- that, and a part of other amino acid sequences are inserted, or 1 or two or more amino acid have combined with the amino terminal and/or the C terminal \*\*\*\* -- or a sugar chain -- the same -- deletion -- or you may permute. [ that a part of the amino acid sequence is permuted by deletion or other amino acid ]

[0008] The collagenolysis accelerator of this invention makes above-mentioned HGF an active principle, and HGF has the operation which promotes decomposition (increment in collagenase activity) of a collagen, as shown in the example of the after-mentioned trial. Therefore, it is useful also to its prevention besides the therapy of the following fibrosing disease failure, and useful to a therapy and prevention of the disease to which collagenase activity fell, for example, the osteopetrosis etc. Moreover, the fibrosing disease failure therapy agent of this invention is useful for the therapy of the fibrosing disease failure characterized by too much fibroblast production of the affinity organization matrix which makes above-mentioned HGF an active principle similarly, and contains a collagen, fibronectin, and glycosaminoglycan (GAG). The following failure is included by this.

[0009] Arteriosclerosis, the chronic glomerulonephritis, skin keloid generation, progressive systemic sclerosis (PSS), hepatic fibrosis, the fibroid lung, the cystic fibrosis, the chronic transplant disease for a host, the scleroderma (a part and whole body), a pay RONI Mr. disease, the fibrosing disease of a phallus, the urethrostenosis after the cystoscopy, the internal adhesion after a surgical operation, the myelofibrosis, an idiopathic posterior part peritoneum fibrosing disease [0010] The collagenolysis accelerator of this invention and a fibrosing disease failure therapy agent are applied to the collagenolysis promotion and the fibrosing disease failure therapy in others and mammals (for example, a cow, a horse, Buta, a sheep, a dog, a cat, etc.). [ Homo sapiens ]

[0011] Although the collagenolysis accelerator of this invention and a fibrosing disease failure therapy agent can take various formulation (for example, liquids and solutions, a solid preparation, a capsule, etc.), let them be injections, inhalations, suppositories, or an oral agent with the support of it and common use of HGF which are generally an active principle. The injections concerned can be prepared with a conventional method, for example, HGF can be filtered with a filter etc., after dissolving in suitable solvents (for example, the sterilized water, the buffer solution, a physiological saline, etc.), and it can sterilize, and they can be prepared by filling up a sterile container subsequently. As an HGF content in injections, it is usually preferably adjusted to 0.001 to 0.1 (W/V



hydroxyproline content (Hyp; index of fibrosis) and collagenase (collagen dialytic ferment) activity in a hepatic tissue were measured by Kivirikko's and others approach (Anal.Biochem, 19, 249, 1967), and Murawaki's and others approach (J.Biochem, 108, 241, 1990), respectively. furthermore, DNA and the protein content in a hepatic tissue -- respectively -- Dishe -- it measured with the strange method (Biochem, J, 62, 315, 1956) and protein assay kit (biotechnology rat company make) by Burton of law. The result is shown in Table 1. Moreover, the Hitachi 7150 mold automatic analyzer analyzed clinical biochemistry inspection of the blood serum which collected blood from back vena cava to coincidence, and was extracted. The blood test measured a platelet count, a white blood cell count, a number of red cell, a hematocrit value, and hemoglobin concentration using multi-item automatic blood cell counters (E-4000, Sysmex) by the EDTA blood extracted from back vena cava. Moreover, plasma coagulation ability (coagulation time by the prothrombin time, the amount of fibrinogens, and a HEPAPURASUCHIN test and a thrombo test) was measured using the automatic coagulation ability measuring device (KC-40) with the plasma which mixed 3.8% sodium-citrate water solution and the blood extracted from back vena cava at a rate of 1:9. The result is shown in Table 2.

[0019]

[Table 1]

表 1

	溶媒投与群	HGF投与群	健常動物
肝重量 (g)	9.33±0.72	13.02±0.53 **	13.59±0.51 **
総DNA量 (mg/肝臓)	33.6±2.5	39.3±1.8 *	44.8±1.7 **
総蛋白量 (g/肝臓)	1.36±0.12	1.84±0.09 **	2.33±0.09 **
コラゲナーゼ活性 ( $\mu$ g/min/g-肝臓)	0.22±0.01	0.36±0.07 **	0.27±0.02
ヒドロキシプリン含量 ( $\mu$ g/g-肝臓)	423.1±35.9	300.1±18.0 **	129.3±6.4 **

平均値±標準誤差 (n=10)

\* : P&lt;0.05, \*\* : P&lt;0.01 溶媒投与群との有意差

[0020]

[Table 2]

表 2

血 液 生 化 学 検 査 値	溶媒投与群	HGF投与群	健常動物
GOT (IU/l)	136±9	78±4 **	64±3 **
GPT (IU/l)	50±4	28±1 **	15±1 **
γ-GTP (IU/l)	4.8±0.4	3.4±0.2 **	1.8±0.1 **
総ビリルビン (mg/dl)	0.45±0.08	0.25±0.01 **	0.19±0.01 **
直接型ビリルビン (mg/dl)	0.20±0.02	0.18±0.00	0.13±0.01 **
総蛋白 (g/dl)	4.9±0.1	6.4±0.1 **	5.7±0.0 **
アルブミン (g/dl)	2.4±0.1	3.1±0.1 **	2.6±0.0 **
血糖値 (mg/dl)	131±4	152±6 **	180±8 **
総コレステロール (mg/dl)	53±2	98±5 **	72±3 **
HDL-コレステロール (mg/dl)	28.0±1.9	61.6±3.9 **	41.3±1.7 **
トリグリセリド (mg/dl)	70±9	152±18 **	157±16 **
リン脂質 (mg/dl)	116±4	208±8 **	160±5 **
βリポ蛋白 (mg/dl)	107±11	222±20 **	202±18 **
血 液 ・ 凝 固 検 査 値	溶媒投与群	HGF投与群	健常動物
血小板数 (10 <sup>4</sup> /μl)	31±5	78±4 **	105±5 **
白血球数 (10 <sup>3</sup> /μl)	144±6	101±8 **	87±8 **
赤血球数 (10 <sup>6</sup> /μl)	667±16	702±9 **	755±6 **
ヘマトクリット値 (%)	38.9±0.9	41.6±0.3 *	44.3±0.3 **
ヘモグロビン濃度 (g/dl)	12.7±0.3	13.6±0.1 **	14.6±0.1 **
プロトロンビン時間 (sec)	15.6±0.5	13.8±0.2 *	13.8±0.2 *
フィブリノーゲン (g/dl)	1.45±0.10	2.05±0.11 **	2.31±0.05 **
ヘパプラスチン時間 (sec)	37.3±2.7	28.6±0.6 **	28.5±0.5 **
トロンボテスト時間 (sec)	30.0±1.9	22.5±0.3 **	22.8±0.3 **

平均値±標準誤差 (n=10)

\*: P&lt;0.05, \*\*: P&lt;0.01 溶媒投与群との有意差

[0021] 2. As shown in a result table 1 and Table 2, by the repeated-dose administration of DMN, by the solvent administration group (contrast), expansion and withering of the fibrosis of a remarkable liver were observed and the fall of a liver function clear from clinical biochemistry, blood, and a coagulation system inspection value was accepted. On the other hand, the liver function test value of an HGF administration group showed the value near a healthy animal with the difference more significant than those values of a solvent administration group, and showed the clear improvement effect. Moreover, DNA in a hepatic tissue, the amount of proteins, and collagenase activity rose intentionally by HGF administration, the hydroxyproline content which is the index of fibrosis fell intentionally, and liver weight was mostly recovered to normal level.

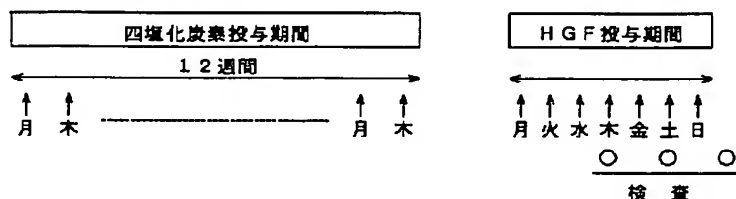
[0022] It administered orally to the operation Wister system male rat (6 weeks old) of HGF to an example 2 carbon-tetrachloride liver fibrosis rat to the moon, the carbon tetrachloride was administered orally to Thursday for 12 weeks by the dosage of 0.7 ml/kg every week, and the liver fibrosis model was created. Two following trials were presented with this carbon-tetrachloride liver fibrosis rat.

[0023] \*\* HGF was administered intravenously to the carbon-tetrachloride liver fibrosis rat (one groups [ 13-14 ]) of the trial A repeated-dose administration trial above by 50 and 500microg/kg between seven days (100, and 1000microg/kg / day) of bis dice. GOT in the blood serum on 3, 5, and

the 7th, GPT, and a  $\gamma$ -glutamyl transpeptidase value change were compared with the solvent administration group (contrast) from HGF administration initiation (following administration schedule 2 reference). The result is shown in drawing 1. As shown in drawing 1, the recovery promotion operation was accepted from the 7th on 100microg/kg / day in 1000microg/kg / day on the 5th.

[0024]

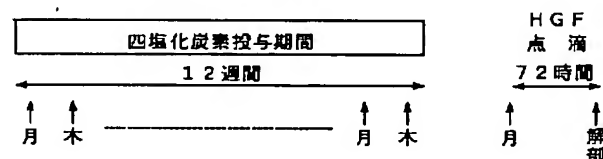
#### 投与スケジュール 2



[0025] \*\* Using the above-mentioned carbon-tetrachloride liver fibrosis rat of trial B instillation trial each 12-13 groups, from the catheter detained in the jugular vein, continuous-drip-infusion impregnation of HGF was carried out at a rate of 100, and a 1000microg/kg / day, and it dissected 72 hours after HGF administration initiation (following administration schedule 3 reference).

[0026]

#### 投与スケジュール 3



[0027] GOT in a blood serum, GPT, total serum protein, and albumin measured the coagulation time of a HEPAPURASUCHIN test with the automatic coagulation ability measuring device (KC-40) about the coagulation ability of the plasma which mixed 3.8% sodium-citrate water solution and the blood extracted from back vena cava at a rate of 1:9 with the Hitachi 7150 mold automatic analyzer. Moreover, the hydroxyproline content (Hyp) in a hepatic tissue was measured by the approach of Kivirikko and others shown above as an index of fibrosis. The result is shown in Table 3 and drawing 2. As shown in Table 3 and drawing 2, the fall of the hydroxyproline content in a hepatic tissue which suggests an improvement of the liver function test value for which HGF depended on the dosage 100microg/kg / above the day, recovery of \*\*\*\*\*, and the improvement of fibrosis was observed.

[0028]

[Table 3]

表 3

	総蛋白量 (g/dl)	アルブミン量 (g/dl)
溶媒投与群	5.0 ± 0.1	1.8 ± 0.1
HGF投与群(100 $\mu$ g/kg/日)	5.5 ± 0.2 *	2.1 ± 0.1 *
HGF投与群(1000 $\mu$ g/kg/日)	5.7 ± 0.2 **	2.1 ± 0.1 *
健常動物	6.0 ± 0.1 **	2.4 ± 0.03 **

平均値 ± 標準偏差 (n = 12 ~ 13)

\* :  $P < 0.05$ , \*\* :  $P < 0.01$  溶媒投与群との有意差

[0029] The survival rate in the HGF administration group and the group non-prescribing a medicine for the patient to the liver fibrosis rat caused by the effectiveness \*\* trial ADMN of HGF to the hepatic insufficiency accompanying the rat liver fibrosis and it which were caused by example 3D MN was examined. The administration schedule of drugs is shown above drawing 3. DMN dissolved in the physiological saline by 1% of concentration was injected intraperitoneally at a rate of 10microl on the day shown by the six-week arrow head of 3 time \*\* \*\* at one week as per



[ DMN ] weight of 1kg of SD system male rat. After DMN administration initiation, from the 21st day, Homo sapiens HGF or a physiological salt solution was administered intravenously every day [ period ] which was shown with the band which carried out hatching, and investigated the survival rate (%) of a trial rat daily. The result is shown in drawing 3 . In drawing 3 , in a dotted line, a physiological-salt-solution administration group (a control group, n= 10) and a broken line show an HGF50microg/kg weight administration group (n= 5), and a continuous line shows an HGF200microg/kg weight administration group (n= 5). As shown in drawing 3 , the survival rate improved by administration of HGF and the example of death was not especially accepted in the HGF200microg/kg weight administration group.

[0030] \*\* The type I collagen deposition (organization view) of rat liver which medicated the bottom of trial BHGF administration or un-prescribing a medicine for the patient with DMN was investigated. That is, for detection of a fibrosis substrate, the rat was killed on the 42nd in the trial shown in drawing 3 , and liver was extracted. Liver was quickly frozen in the OTC compound, and after making the created intercept react with a rabbit origin anti-rat collagen type I antibody (product made from LSL), and a fluorescence labeling goat origin anti-rabbit IgG antibody, it performed photograph shooting of a sample in the immunofluorescent stain of a collagen. The result is shown in drawing 4 . As shown in drawing 4 , there was a significant difference in the deposition of the type I collagen in liver. In the contrast rat of a physiological-salt-solution administration group, the deposition in the liver of a type I collagen is clear, and the east of a thick or thin type I collagen fiber was accumulating it on the blood vessel or the perimeter of hepatocyte extensively. These views disappeared on the dosage dependence target by administration of HGF, and the improvement of leaflet structure was more remarkable in the HGF administration group. Thus, the liver fibrosis prevention effectiveness of HGF became clear from the organization view of a liver intercept.

[0031] \*\* Change of the prothrombin time by trial CHGF administration, a hepatocyte deviation enzyme, and a liver hydroxyproline content was investigated. That is, it dealt with the rat according to the design of experiment shown by drawing 3 . The prothrombin time (PT), albumin (Alb), glutamic oxaloacetic transaminase (GOT), In the value in glutamate pyruvate transaminase (GPT) and the blood serum (plasma) of alkaline phosphatase (ALP), and a list, a liver hydroxyproline content (HYP) A healthy rat group (in n= 5 and Table 4, it is displayed as a healthy rat), the group which carried out DMN processing for five weeks, and prescribed only a physiological salt solution for the patient (in n= 5 and Table 4, it is displayed as 5W), The group which carried out DMN processing for six weeks, and prescribed only a physiological salt solution for the patient (in n= 1 and Table 4) HGF was measured instead of 6W, the display, or a physiological salt solution, respectively by the group prescribed for the patient in 50microg/kg weight (it is displayed as 50 in n= 3 and Table 4), and 200microg/kg weight (it is displayed as 200 in n= 5 and Table 4). The result is shown in Table 4. In addition, front Naka and the value displayed as 5W are values which slaughtered and obtained the rat on the 35th, and values other than this can slaughter a rat after DMN treatment initiation on the 42nd. As shown in Table 4, it became clear by administration of HGF that fibrosis of liver and relaxation of hepatic insufficiency were achieved.

[0032]

[Table 4]

表 4

			PT (sec.)	Alb (mg/dl)	GOT (IU/L)	GPT (IU/L)	ALP (IU/L)	HYP (μg/g Liver)
健康ラット	(n=5)		14.2 ± 2.7	4.5 ± 0.04	76 ± 16	19 ± 3	459.6 ± 65	224 ± 89
生理食塩液	5W	(n=5)	—	—	314 ± 71	132 ± 16	1967 ± 236	740 ± 211
	6W	(n=1)	80<	2.6	162	49	1288	1011
H G F	50	(n=3)	32.0 ± 11.2	2.6 ± 0.8	165 ± 38	42 ± 14	1081 ± 523	789 ± 58
	200	(n=5)	21.4 ± 5.2	3.1 ± 0.7	164 ± 38	40 ± 12	1053 ± 448	666 ± 116

[0033] To SD system male rat of the 5 weeks old of the liver fiber reduction effectiveness \*\*

approaches by the HGF administration by the example 4DMN liver fibrosis rat, DMN was injected intraperitoneally for four weeks 3 times (the moon, fire, water) per week by the dosage of 10microl/kg, and the liver fibrosis rat was produced to it. This rat was medicated with HGF of 1 mg/kg, or the solvent (HSA 2.5mg/ml, Tween 80 phosphate buffered saline containing 0.01%) of 1 ml/kg from the time of the 5 times (moon, fire, water, tree, gold) DMN administration initiation per week, and a medicine was prescribed for the patient till the 4th for the 4th week. The rat was slaughtered on the 5th for the 4th week, liver was fixed to the neutral formalin after extraction, and the Masson trichrome stain which dyes the fibrous tissue in various colors after intercept production was given. In addition, the liver of a healthy rat was similarly dyed for the comparison. About the liver preparation of the dyed each object, using image-analysis equipment (T.Watanabe et al.Analytical Cellular Pathology 4 (3), 248, 1992), the area of an organization which carried out fibrosis in the whole surface product of an organization intercept was calculated, and extent of fibrosis was measured. In addition, the population used for analysis was six except the individual which HGF neutralization activity generated in the blood serum about eight healthy rats, eight DMN+ solvent administration groups (DMN), and a DMN+HGF administration group (HGF+DMN).

[0034] \*\* The result result was shown in drawing 5 . Each point in drawing shows the rate of the fibrous tissue of the each object liver of a healthy rat (n= 8) and DMN administration rat (n= 8) and a DMN+HGF administration rat (n= 6) at percent. Moreover, the bar of striping shows the average. As shown in drawing 5 , as compared with the healthy rat, the rate of the fibrous tissue under liver tissue increased to 7.3% to an average of 1.3% by DMN. On the other hand, in DMN+HGF, it fell to 2.8% and the liver fibrosis mitigation by DMN was shown by administration of HGF.

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[Translation done.]

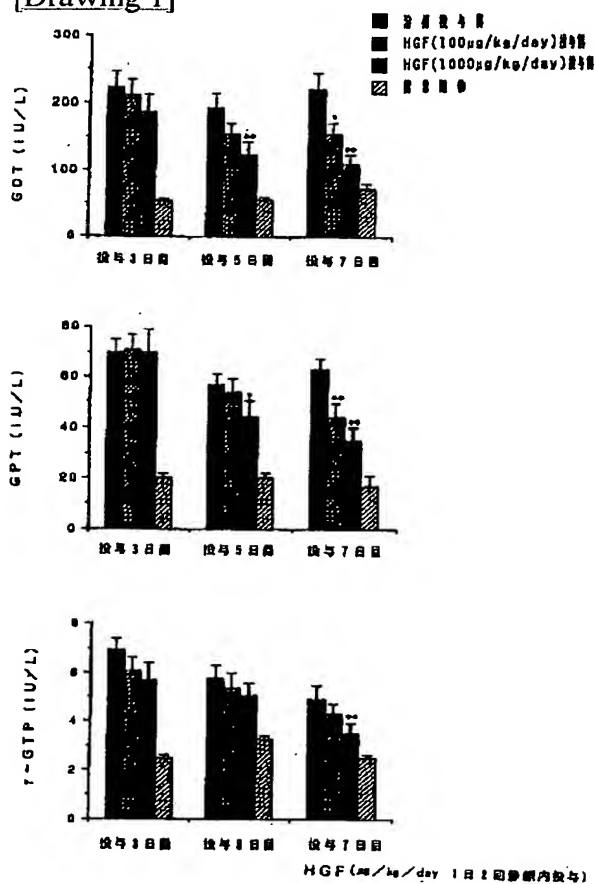
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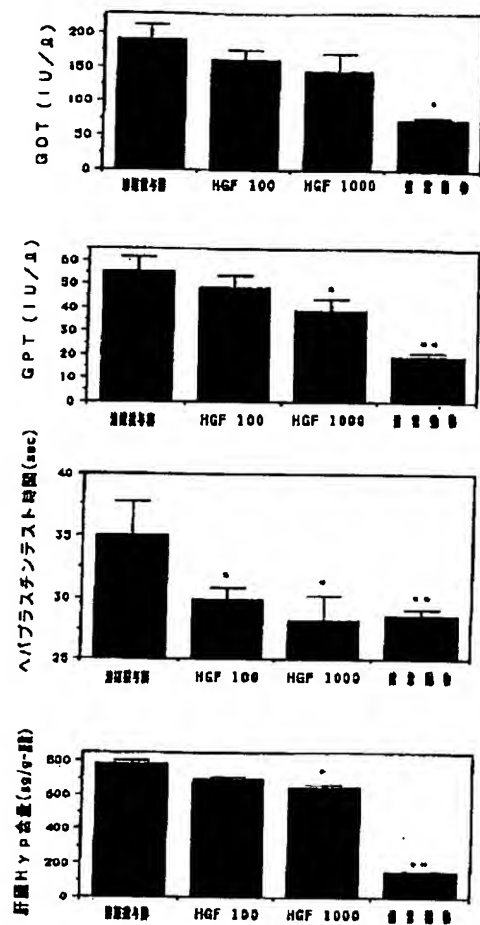
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3. In the drawings, any words are not translated.

## DRAWINGS

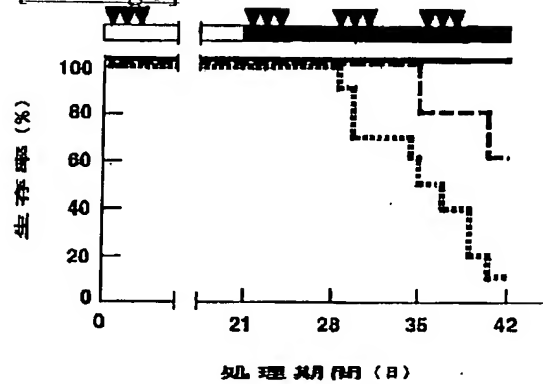
[Drawing 1]



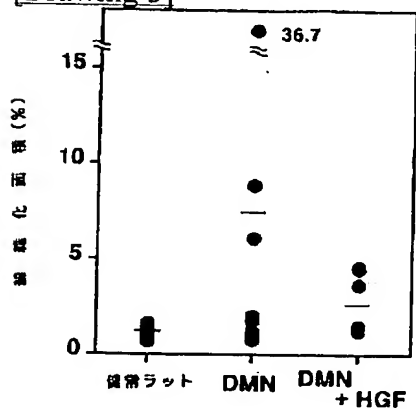
[Drawing 2]



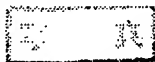
[Drawing 3]



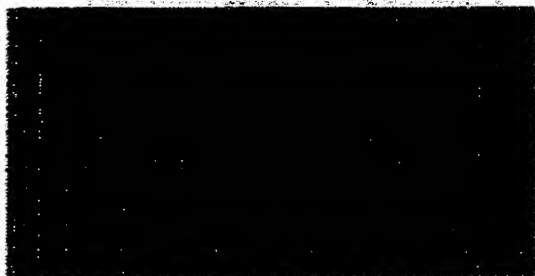
[Drawing 5]



[Drawing 4]



図面代用写真

HCF (200  $\mu$ g/kg体重)HCF (50  $\mu$ g/kg体重)

生理食塩液



健康ラット

[Translation done.]

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